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Family studies of congenital heart block associated with Ro antibody

WILHELMINA M H BEHAN,* PETER O BEHAN,† J M REID,‡ W DOIG,§ JAN GAIRNS†

*From the Departments of *Pathology and †Neurology, Glasgow University; ‡Department of Cardiology, Western Infirmary; §Department of Paediatric Cardiology, Royal Hospital for Sick Children, Glasgow*

SUMMARY Complete congenital heart block is associated with the presence of maternal autoantibodies to small ribosomal nucleoproteins (such as anti-Ro) which cross the placenta and may be deposited at the site of cardiac damage. Ten such cases of congenital heart block, their mothers, and their siblings were studied. The seropositive mother of one case had a similar conduction defect (bifascicular block) to that in her affected child. None of the siblings examined had cardiac lesions. Six mothers had Ro or La antibody five to 17 years after the birth of the affected child. Four mothers examined 11–32 years after the birth of an affected child were seronegative. Three of these mothers had evidence of a connective tissue disorder. This evidence is consistent with a hypothesis that a maternal viral infection, associated with autoantibody production, leads to virus crossing the placenta, damaging the fetal heart, and eliciting local deposition of maternal antibody.

The development of complete congenital heart block is associated with the transplacental passage of maternal autoantibodies directed at small ribosomal nucleoproteins.[1–3] Most mothers who give birth to an affected infant have anti-Ro or the related anti-La serum antibody, as do their infants up to three months after birth.[1 3 4] Immunoglobulins thought to be of maternal origin were found at the site of cardiac damage in one baby who died three days after birth.[2] A mother who gives birth to such an infant may or may not have a connective tissue disease at the time, but 30–60% of these women will develop such an illness subsequently.[1–5] There are, however, several unanswered questions—for example, what is the risk of subsequent offspring being affected? Are the two sexes at equal risk (affected males tend to have complete congenital heart block only while affected females show other stigmata of the neonatal lupus syndrome[3]), and, because the antibody seems to produce such severe damage to fetal myocardium, what is its long term effect on adult heart tissue?

To try and answer these questions we investigated 10 individuals who had this isolated cardiac abnormality and their mothers.

Requests for reprints to Dr Wilhelmina M H Behan, Department of Pathology, Western Infirmary, Dumbarton Road, Glasgow G11 6NT.

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Patients and methods

CASES OF COMPLETE CONGENITAL HEART BLOCK

Table 1 gives the clinical details of the 10 cases (five male and five female patients). Six of them had had permanent pacemakers inserted. One was diagnosed in utero, four were identified in the neonatal period, and the others at ages from 18 months to 20 years. They are currently 5–32 years old.

DETECTION OF ANTIBODIES

Serum samples were stored at −70°C until use and then heat inactivated before testing. Antinuclear and anti-DNA antibodies were sought by standard indirect immunofluorescence techniques, with rat liver and *Crithidia luciliae* as the respective substrates.[6 7] Rheumatoid factor was assayed by a commercial latex agglutination test (Rheumaton) followed with an enzyme linked immunoadsorbent assay of positive samples for IgM. Antibodies to soluble cellular antigens were detected by means of saline extracts of human spleen (a potent source of Ro antigen and the other nucleoprotein complexes—Sm and nuclear ribosomal nucleoprotein (nRNP)) and fresh calf thymus (for the detection of La (SS-B)), prepared as described.[8] The serum was screened first by double immunodiffusion and then by counterimmunoelectrophoresis.[9] Known control antisera were incorporated; all positive results were confirmed by

Table 1 *Clinical data on 10 cases of complete congenital heart block*

	Patient									
Data	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Sex	M	F	M	M	F	F	M	M	F	F
Age at diagnosis	Neonate	Neonate	Neonate	In utero	18 mnth	5 yr	Neonate	3 yr	20 yr	17 yr
Pacemaker inserted	Yes	Yes	Yes	No	Yes	No	No	No	Yes	Yes
Present age (yr)	5	7	10	10	17	12	16	17	21	32

the demonstration of a complete reaction of identity with one of the positive controls, obtained with the help of Dr P J Maddison, Dr G R V Hughes, and the Center for Disease Control, Atlanta; the center also supplied anti-La, anti-nRNP, and anti-Sm antisera (antisera to related nuclear antigens). Control sera were obtained from a group of 40 pregnant women, age-matched for the mothers tested.

The Ouchterlony plates were examined at 24, 48, 72, and 96 hours. The counterimmunoelectrophoresis plates were left overnight at room temperature, then washed in 5% citrate for four hours, in saline for 48 hours, and stained with Coomassie blue.

Results

Table 2 shows the results of examining the mothers. Their ages at the time of birth of the affected child ranged from 22 years to 43 years. They are currently from five to 32 years older. Five have a connective tissue disease (1 dermatomyositis and 4 rheumatoid arthritis). The mothers of cases 1 and 5 developed severe dermatomyositis and rheumatoid arthritis respectively before any of their children were born. The other mothers (of cases 8, 9 and 10) developed arthritis after the birth of the affected infant. The mother of case 7 had rheumatic fever aged 12 years and since then has taken prophylactic antibiotics before any dental procedure.

Only the mother of case 1, who has been taking prednisolone 5 mg per day for the past 12 years, complained of cardiac symptoms—she had exertional dyspnoea. Detailed questioning elicited no complaints from the other mothers. None was hypertensive. Eight underwent electrocardiography and echocardiography. The mother of case 8 was too anxious to attend for investigation while the mother (aged 71 years) of case 10 had fallen and was unable to attend the hospital.

Table 2 *Data on the mothers of 10 cases of complete congenital heart block*

	Mother of case:									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Mother's age at birth of affected child	27	29	25	31	22	25	39	43	25	39
Mother's present age with interval since the birth in brackets	32 (5)	38 (7)	35 (10)	41 (10)	39 (17)	36 (11)	55 (16)	60 (17)	46 (21)	71 (32)
Maternal illnesses	Dermatomyositis for 15 yr	—	—	—	Mild rheumatoid arthritis for 18 yr	—	Rheumatic fever aged 12 yr. Healthy since then	Moderately severe arthritis for 10 yr	Rheumatoid arthritis for a yr	Rheumatoid arthritis for 15 yr
Cardiac findings/ symptoms	Exertional dyspnoea	—	—	—	—	—	—	—	—	—
Blood pressure (mm Hg)	130/80	110/80	130/80	150/95	120/70	110/70	145/90	Not done	124/90	Not done
Electrocardiogram and echocardiogram	Normal	Bifascicular branch block	Normal	Normal	Normal	Normal	Normal	Not done	Normal	Not done
Antibodies present to Ro, La, antinuclear (ANA), rheumatoid factor (RhF)	Anti-Ro, ANA 1/1000, RhF +ve, rheumatoid IgM 1500 units/ml	Anti-Ro	Anti-Ro, RhF +ve	Anti-Ro, ANA 1/64, RhF +ve	Anti-Ro	None	Anti-La, ANA 1/1000, anti-DNA +ve	None, RhF +ve	None, RhF +ve	None

Table 3 *Family relationships in individuals with complete congenital heart block*

	Case No									
	1	*2**	*3*	*4*	*5**	*6**	*7*	*8*	*9**	*10*
Present age (yr)	*5*	*7*	*10*	*10*	*17*	*11*	*16*	*17*	*21*	*32*
Birth order	Youngest of 3	Second of 3	Younger of 2	Younger of 2	Elder of 2	Elder of 2	Youngest of 3	Youngest of 6	Third of 4	Youngest of 3
Siblings	Sister aged 11. Brother aged 10	Brother aged 10. Brother aged 18 mnth	Sister aged 13	Brother aged 13	Brother aged 16	Sister aged 8	Brother aged 28. Brother aged 25	Sisters aged 33, 31, and 27 yr, then miscarriage. Brother aged 23	Sister aged 26, then still-born anence-phalic. Sister aged 20	Brother aged 43, then miscarriage

*Subsequent normal delivery.

The only abnormality detected was in the mother (aged 38) of case 2, who was a non-smoker and who had partial right bundle branch block with left axis deviation. She had no evidence of ischaemic heart disease. Five mothers had Ro antibody, when tested five to 17 years after delivery and one had La antibody, at 16 years after delivery. Four mothers, examined at 11 to 32 years later, were seronegative. Two years previously the same results had been found. Six mothers had a connective tissue disease with positive serology. The mothers of cases 1 and 7 had high titres (1/1000) of antinuclear antibodies, and the mother of case 7 also had DNA antibodies, indicative of systemic lupus erythematosus. Five mothers (of cases 1, 3, 4, 8, and 9) had sera positive for rheumatoid factor. None had antibodies to the related extractable nuclear antigens, Sm or nRNP.

The affected individuals (cases 5 to 10) were themselves tested for the same panel of antibodies and were, as expected, all negative.

Table 3 shows the family relationships. Each case of congenital heart block had one or more siblings. The older ones had been examined by a cardiologist at the time the index case was diagnosed and none was found to have any evidence of cardiac disease. Four families had children born after the affected child, from one to eight years later, and all of these had undergone cardiac evaluation with electrocardiograms and had been found to be normal. The other six patients were the youngest in their families; the births of three of them were preceded by miscarriages.

We looked at three generations in the family of one patient (case 9) who was herself seronegative as expected. She recently gave birth to a healthy son.

Discussion

Complete congenital heart block is a rare cardiac lesion, occurring as an isolated finding in approximately 15 of 20 000 live births,[10] but it accounts for one in 200 referrals to specialised cardiac centres and is of considerable importance since those affected require lifelong cardiac supervision, usually with the insertion of a series of pacemakers.[10] Although the condition was originally described in 1901[11] and the first case of an affected infant with a mother who had systemic lupus erythematosus was reported in 1945,[12] it was not until 1977 that the consistent relation of this heart lesion to maternal connective tissue disease was established.[13] Weston *et al* then suggested that the SS-A (Ro) antibody might be a serological marker for the neonatal lupus syndrome, including congenital heart block.[14] The suggestion was quickly confirmed by work showing that 34 of 41 mothers who had an affected infant had anti-Ro serum autoantibody, as did seven of eight of their infants.[1] Half of the mothers also had the related La antibody,[1] which may be exclusively associated with Ro but no other autoantibodies.[3] Since then, it has been postulated that the maternal autoantibody actually causes the cardiac damage, because maternal immunoglobulin, with or without complement, was found in the hearts of affected infants.[2 15]

Two questions immediately arise. What is the risk to the child of a mother who is seropositive for anti-Ro and what may be the long term effect of the antibody on the mother herself? In attempting to answer them, we concluded that the antibody itself cannot be the damaging agent although it may provide a clue to the pathogenesis.

Our group of mothers was typical of those previously described, with specific antibodies in six mothers and three of the four seronegative women having developed a connective tissue disease. They were tested 11–32 years after the birth of an affected child, which may explain their seronegativity. One mother did not have any positive immunological results but she is only 36 years old.

An unexpected finding, not previously recorded in any such mothers, was that of partial right bundle

branch block with left axis deviation, indicating bifascicular conduction damage, in the mother of case 2. She was an energetic, normotensive woman of 38, a non-smoker, with no evidence of ischaemic heart disease but this result showed that she had evidence of conduction damage similar to that of her middle son. None of the other seven women had any abnormality, although the connective tissue disorders, which four of them had, are known to be associated with such cardiac lesions. These were dermatomyositis,[16 17] rheumatoid arthritis,[18] and systemic lupus erythematosus.[19] It is plain, therefore, that even in predisposed mothers, the Ro antibody may be present for up to 17 years without causing any injury to the maternal conduction system.

As for the risk to the child of a seropositive mother, all our cases had unaffected siblings—older, younger, or both. Four children, the younger siblings of cases 2, 5, 6, and 9 were undoubtedly exposed to maternal Ro antibody, while others (of cases 1, 8, 9, and 10) are likely to have been at risk. The mother of case 1 had had severe dermatomyositis (a disorder in which Ro antibodies may appear[17]) since before the birth of any of her three children, while the other mothers were each delivered of a stillbirth immediately before the birth of their affected infant. Ro antibody is undoubtedly associated with fetal loss.[1 20] In summary, the ten mothers had a total of 30 pregnancies with at least 18 fetuses exposed to Ro antibody: 10 developed heart block, three died, and five were unaffected healthy infants.

Similar anomalous findings have been reported before. They include the birth of dizygous twins, one affected and one not, and three healthy mother and infant anti-Ro seropositive pairs among 300 randomly selected pregnancies.[1 13 21] The explanations advanced previously have included possible fluctuations in antibody titre, differing maternal-fetal histocompatibility antigens[3 22] and a hormonal effect, because female infants are more commonly affected,[3 4] but none of these has been substantiated.

The most telling facts against Ro antibody being the cause of the damage, however, are that first, the antigen against which it is directed is present not only in cardiac conduction tissue but in virtually every mammalian cell,[23] including fetal tissue at 16–18 weeks' gestation[24] (the earliest stage at which heart block has been detected). Secondly, since Ro is intracellular it should not elicit an immunemediated attack—although there is a possibility that it could appear on Purkinje cell membranes at a critical developmental stage.[24]

We would like to suggest a simple explanation for the anomalies in our study and those of others, namely that, before or during the early stage of pregnancy, the mother sustains a mild, perhaps unrecognised viral infection, to which she produces Ro or La antibodies. The virus crosses the placenta and damages the infant myocardium while specific maternal antibody, also crossing the placenta, localises in the same area. The infection in the child is self-limiting but it may persist in the mother so that a series of infants are affected. The mother's own heart may also be damaged (see case 2). An obvious viral candidate is a Coxsackie virus, since these viruses are common causes of myocarditis,[25] show considerable sequence homology with certain intracellular proteins,[26] and may persist in connective tissue diseases—for example dermatomyositis[27] (see case 1).

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